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PP-159 [Bee Health]

How does combined exposure to pesticides and *Nosema ceranae* affect bee health?

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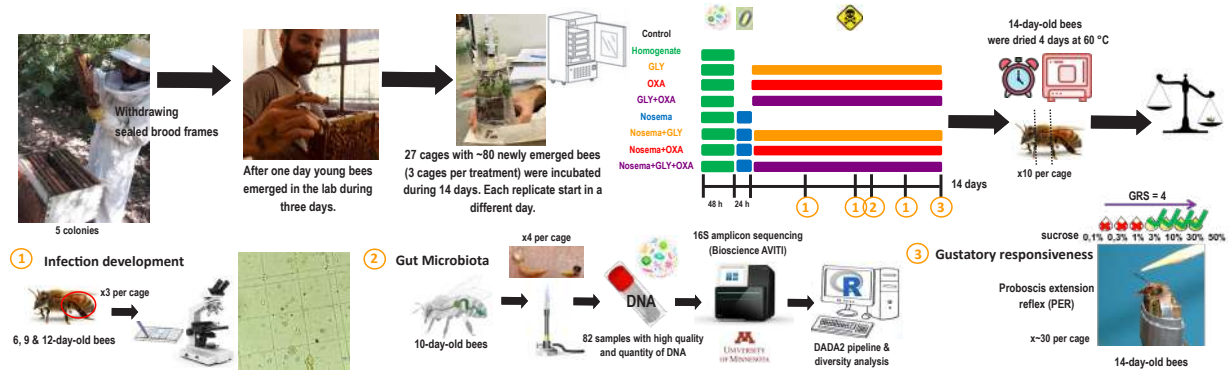
INTRODUCTION

Agricultural intensification in recent decades has led to extensive use of agrochemicals. Beekeeping is not exempt from this problem. The demand for pollination services has increased, and beekeepers must frequently apply acaricides to control *Varroa destructor.* Therefore, after pesticide applications on crops or hives, growing residues have been detected in honey and beebread[1]. Also, pests and pathogens are more probable in crowded apiaries with honey bee colonies in stressful environments. In this scenario, the honey bees are chronically exposed to mixtures of pesticide residues, which weaken colonies on a stress treadmill. The question is how the early ingestion of pesticides can alter the development of gut defensive barriers, such as the microbiota, or digestive diseases, such as nosemosis. Hence, the current study aimed to assess the interaction between *Nosema ceranae* infection and a common pesticide mixture. On the one hand, the herbicide glyphosate (GLY), the pesticide most widely applied in crops, has shown detrimental effects in both brood and adult bees[2,3,4]. On the other hand, the organic acaricide oxalic acid (OXA) is one of the most applied options in Latin America. Previous studies indicate that both pesticides have antimicrobial action on the gut microbiota of bees[5,6]. This antimicrobial action is relevant due to the potential detrimental effect that they would exert on the bee health. Adult bees have a gut microbiota consisting mainly of bacteria belonging to 9 genera such as Lactobacillus, Snodgrassella, Gilliamella and Bifidobacterium, among others[6]. These symbionts play a crucial role in nutrition and immunity, acting as barriers against pathogens and pollutants[6]. Therefore, the early disruption of the gut microbiota in young bees would affect their physiology or the physiopathogenesis of digestive diseases such as nosemosis. Moreover, the first weeks in the lifespan of adult bees are crucial for the survival of the colony because they take care of the queen and the next generations of larvae.

METHODS

We caged around 80 newly emerged bees per treatment (3 replicates with bees from 5 colonies) and reared them under lab conditions (31 ºC and 60% RH). Then, we inoculated the caged bees with native gut microbiota by administering 2 mL of homogenate with gut content of nurse bees from hives and sucrose solution (5 guts : 1 mL PBS 1X : 1 mL sucrose sol. 2:1) during the first two days in all treatments, besides a control group without homogenate. Furthermore, the adult bees were chronically exposed to the herbicide glyphosate (GLY: 1.6 mg/L), the acaricide oxalic acid (OXA: 280 mg/L) or both administered in the sucrose sol. (1:1) at 3 days of age, besides a group without pesticides (homogenate). These pesticide concentrations correspond to the maximum value of residues detected in hives according to the literature[5]. The same 4 treatments were performed adding spores of *N. ceranae* (80.000 spores/bee) in 1 mL of sucrose sol. (1:1) during 24 h at 2 days of age. Therefore, we carried out 9 treatments with the different possible combinations of exposures, measuring intake and survival. Then, the infection development was monitored in 6, 9 and 12-day-old bees while the diversity of the microbiota was analysed after 10 days. Lastly, we assessed physiological alterations in 14-day-old bees, measuring dry weight and gustatory responsiveness[2,4].



RESULTS

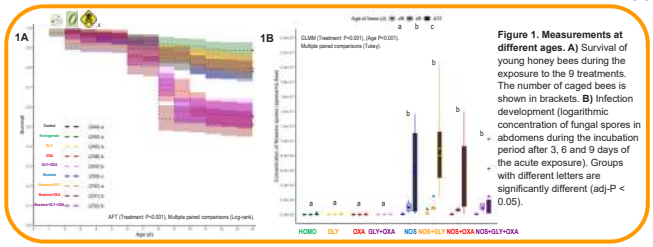


Figure 1. Measurements at different ages. A) Survival of young honey bees during the exposure to the 9 treatments. The number of caged bees is shown in brackets. **B)** Infection development (logarithmic concentration of fungal spores in abdomens during the incubation period after 3, 6 and 9 days of the acute exposure). Groups with different letters are significantly different (adj-P < 0.05).

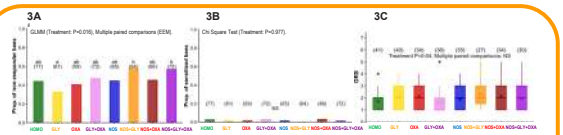


Figure 3. Physiological markers in 14-day-old bees. The sucrose response threshold and dry weight of the young honey bees were assessed after ingesting 8 different combinations of the two pesticides and spores of *Nosema ceranae*. **A)** Proportion of non-responder bees, i.e. without PER after stimulation with water or sucrose solution. Groups with different letters are significantly different (adj-P < 0.05). **B)** Proportion of sensitized bees, i.e. with non-specific PER to water or sucrose solution. **C)** Dispersion of the gustatory response score (GRS) of each bee with specific PER to sucrose solution (CLM followed by EMM). The number of assessed bees is shown in brackets. Dry weight showed no significant differences among treatments (GLMM, P=0.407).

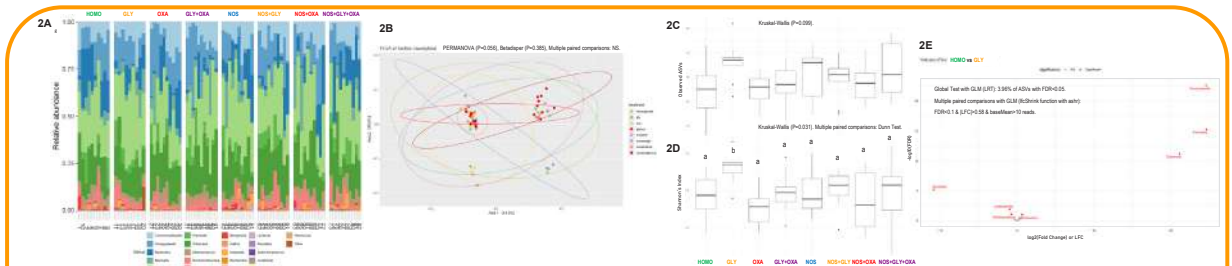


Figure 2. Diversity Analysis of Gut microbiota of 10-day-old bees. A) Relative abundance of bacterial genera in each sample (1 gut) per treatment plotted with stacked bars after quality control filtering of the dataset. Each bar (bacterial genus) is plotted with different colours (see legend). **B)** Multivariate analysis of beta diversity. PCoA plot is based on distance matrices of Unweighted Unifrac similarities. The points (each sample) are plotted with different colours per treatment (see legend). The ellipses indicate bidimensional confidence intervals (95%) for each treatment. **C&D)** Dispersion of indices of alpha diversity per treatment plotted with boxplots. C) Observed ASVs as an indicator of bacterial community richness in each simple (high index, high richness). D) Shannon´s index as an indicator of bacterial community uniformity in each sample (high index, high uniformity). Groups with different letters are significantly different (adj-P < 0.05). **E)** Differential abundance analysis with DESeq2. Volcano plot of ASVs with a significant difference in the number of reads between homogenate and GLY groups, as an example.

CONCLUSIONS

Our results indicate that exposure to field-relevant concentrations of OXA and GLY showed lethal and sublethal effects on gut microbiota diversity, respectively. Furthermore, the infection with *Nosema ceranae* reduced the survival of young honey bees. However, the pesticides did not interact synergistically with the fungal infection in survival, but the combination of GLY and *Nosema ceranae* slightly affected the sensitivity to sucrose. Therefore, a combination of stressors should not necessarily generate an expectation of a greater deleterious effect because of the complex interaction *in vivo*.

References

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Agricultural intensification in recent decades has led to extensive use of agrochemicals. In fact, beekeeping is not exempt from this problem, and acaricides must frequently be applied to control Varroa destructor. Diseases caused by different pests and pathogens are more probable in crowded apiaries with honey bee colonies in stressful environments. In this scenario, the honey bees are chronically exposed to mixtures of pesticide residues which weaken the colonies on a stress treadmill. Therefore, the question is how the early ingestion of pesticides can alter the development of gut defensive barriers like the microbiota or digestive diseases such as nosemosis. Hence, the current study aimed to assess the interaction between Nosema ceranae infection with a common pesticide mixture. For that, we caged newly emerged bees (3 replicates with bees from 5 colonies) and reared them under lab conditions. Adult bees were chronically exposed to the herbicide glyphosate (0.7 mg/L), the acaricide oxalic acid (280 mg/L) or both administered in sucrose solution (1:1), besides a group without pesticides. According to the literature, these concentrations correspond to the maximum value of residues detected in hives. Meanwhile, the same 4 treatments were performed, adding N. ceranae (80.000 spores/bee) at 2 days of age. Therefore, we carried out 8 treatments with the different possible combinations of exposures, measuring intake and survival. Then, the infection development was monitored at 6, 9 and 12 days of age while the gut microbiota was analysed at 10 days. Lastly, we assessed physiological alterations in 14-day-old bees, measuring dry weight and gustatory responsiveness. The experiment was performed twice, at the beginning and end of the queen oviposition period (early spring and late summer). Our results showed no synergic interactions between the combinations of stressors on survival. However, the pesticides had lethal effects depending on the brood cohort (e.g. oxalic acid showed high mortality in late summer bees). Finally, we found signs of gustatory desensitization when more stressors were combined. Future studies must assess more mixtures and integrate different sub-lethal effects (e.g., in microbiota and behaviour) for a greater understanding of the scale of the bee health impairment.